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The effect of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 expression in association with prostaglandin E_2

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Abstract

We demonstrated previously that endothelin-1 (10^{-14} to 10^{-8} M) promotes lipopolysaccharide-induced cyclooxygenase 2 expression and prostaglandin E_2 production through endothelin ET_B receptors effects which are up-regulated by lipopolysaccharide. In the present study, we confirmed these findings and showed that prostaglandin E_2 (10^{-6} to 10^{-5} M) inhibited the lipopolysaccharide plus endothelin-1-induced cyclooxygenase 2 expression more profoundly as compared to its inhibition of the lipopolysaccharide-induced cyclooxygenase 2 expression. The endothelin ET_B receptor selective antagonist, *N-cis*-2,6-dimethylpiperidino-carbonyl-L-γ-methyl-leucyl-D-L-methoxycarbonyl-tryptophanyl-D-norleucine (BQ788), partly inhibited this suppression. Interestingly, the expression of endothelin ET_B receptors in macrophages was increased by lipopolysaccharide plus prostaglandin E_2 (10^{-8} to 10^{-5} M) about 1.6-fold compared with that evoked by lipopolysaccharide stimulation alone. We also showed that treatment with endothelin-1 at 10^{-14} M (15 min) elevated an intracellular cyclic AMP concentration in macrophages stimulated by lipopolysaccharide or lipopolysaccharide plus prostaglandin E_2 (10^{-6} M) for 6 h, and the elevation in the latter cells was more pronounced. These results suggested that endothelin-1 shows an opposite modulation of lipopolysaccharide-induced cyclooxygenase 2 expression in macrophages through endothelin ET_B receptors, depending on the level of extracellular prostaglandin E_2, and the changes of intracellular cyclic AMP by endothelin-1 may be involved in this mechanism.

Keywords: Endothelin-1; Cyclooxygenase 2; Prostaglandin E_2; Lipopolysaccharide; Macrophage

1. Introduction

Endothelin-1 is one of the three homologous peptides (endothelin-1, -2, and -3) that display a wide variety of biological activities (Yanagisawa et al., 1988; Masaki et al., 1992). Numerous studies have shown that endothelin-1 acts as a potent mitogen toward various cell types including monocyte/macrophages. In addition, macrophages have the capacity of producing endothelin-1 under lipopolysaccharide stimulation (Ehrenreich et al., 1990; Chanez et al., 1996). We recently studied the role of endothelin-1 on inflammatory responses in mouse peritoneal macrophages and showed that at high concentrations of 10^{-11} to 10^{-8} M, it induced cyclooxygenase 2 protein expression and prostaglandin E_2 production in a concentration-dependent manner, an effect which was mediated through mainly endothelin ET_B receptors (Shimada et al., 1998). We also showed that at lower concentrations of 10^{-14} to 10^{-12} M, it promoted lipopolysaccharide-induced cyclooxygenase 2 expression and prostaglandin E_2 production, in which lipopolysaccharide-induced up-regulation of endothelin ET_B receptors and their related process may be involved (Shimada et al., 1999).

Cyclooxygenase 2 is an inducible isoform of cyclooxygenase implicated in inflammatory responses (Smith et al., 1996) and is associated with the production of prostaglandins under pathological conditions. In addition, cyclooxygenase 2 is required for the delayed synthesis of prostanoids by proinflammatory stimuli (Murakami et al., 1994; Reddy and Herschman, 1994; Morham et al., 1995). Prostaglandin E_2 is known to be one of the major modulators derived from cyclooxygenase 2 activation to increase cyclic AMP in macrophages. Recently it has been demonstrated that prostaglandin E_2 exerts feedback regulatory suppression of lipopolysaccharide-induced cyclooxygenase 2 expression through its driven cyclic AMP-related mecha-

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nisms (Pang and Hoult, 1997). In the present study, we tried to clarify the effect of endothelin-1 on cyclooxygenase 2 expression in association with its major reaction product, prostaglandin E_2, in lipopolysaccharide-stimulated macrophages. We estimated here an opposite modulation by endothelin-1 of lipopolysaccharide-induced cyclooxygenase 2 expression depending on the level of extracellular prostaglandin E_2. Moreover, we suggested that the up-regulation of endothelin ET_B receptors by lipopolysaccharide or lipopolysaccharide plus prostaglandin E_2, and the elevation of intracellular cyclic AMP might contribute to this modulation by endothelin-1.

2. Materials and methods

2.1. Materials

The following commercial preparations were used in the present study: lipopolysaccharide (*E. coli*, 055:B5) and endothelin-1 from Peptide Institute (Kyoto, Japan), the endothelin ET_B receptor selective antagonist, *N-cis*-2,6-dimethylpiperidino-carbonyl-L-γ-methyl-leucyl -D-L -methoxycarbonyl-tryptophanyl-D-norleucine (BQ788) from Banyu (Tokyo, Japan), antibody against cyclooxygenase 2 from Cayman (MI, USA) and antibody against human endothelin ET_B receptor from Immunobiological (Gunma, Japan). Enzyme immunoassay kits for prostaglandin E_2 and cyclic AMP were obtained from Cayman.

2.2. Preparation of mouse peritoneal macrophages

This study was performed in accordance with The Japanese Pharmacological Society Guide for the Care and Use of Laboratory Animals. The local Animal Care Committee approved all procedures at Nara Medical University.

Brewer's thioglycolate medium (4.05%, *W/V*) (Nacalai Tesque) was injected i.p. in 10-week-old C57 Black/6 mice (Kiwa Experimental Laboratory Animal, Wakayama, Japan), and then peritoneal exudate cells were collected on day 4 by washing the cavity with 10 ml of ice-cold sterile heparinized Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline. The collected cells were immediately centrifuged at 4°C and the supernatants were discarded. Peritoneal cells were immediately seeded in 12-well plates in 1 ml of Dulbecco's Modified Eagle's Medium (Gibco-BRL, France) containing 10% fetal bovine serum (Nacalai Tesque). After a 90 min incubation at 37°C with 5% CO_2, almost all adherent cells were macrophages, as assessed by measurement of their esterase activity. Then the adherent cells were suspended at 1.0×10^6 cells/ml in medium and seeded in 12 well plates (1 ml/well). Cell viability throughout the experiment exceeded about 95% with trypan blue dye exclusion and measurement of lactate dehydrogenase in cell supernatants with a lactate dehydrogenase–UV test kit (Wako, Tokyo, Japan).

Adherent macrophages were washed with sterile Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline warmed to 37°C and stimulated in the medium by lipopolysaccharide at 10 μg/ml plus endothelin-1 at 10^{-15} to 10^{-8} M for 12 h. After incubation, prostaglandin E_2 concentration in the supernatants was measured by enzyme-linked immunoassay kit, and cyclooxygenase 2 and endothelin ET_B receptor protein expression in macrophages was measured by sodium dodecyl sulfate-polyacrylamide gel/immunoblotting and densitometric analysis.

In the experiment for the effects of BQ788, macrophages were pretreated with BQ788 at 10^{-7} M for 30 min (Mihara et al., 1994), and were then stimulated by lipopolysaccharide at 10 μg/ml alone, lipopolysaccharide plus endothelin-1 (10^{-14} M), or lipopolysaccharide, endothelin-1 plus prostaglandin E_2 (10^{-6} M) for 12 h. Prostaglandin E_2 concentration and the expression of cyclooxygenase 2 and endothelin ET_B receptor protein were measured.

In the experiment for the effect of prostaglandin E_2 on cyclooxygenase 2 expression in macrophages, cells were stimulated by lipopolysaccharide plus prostaglandin E_2 (10^{-8} to 10^{-5} M) in the presence or absence of endothelin-1 (10^{-14} M) for 12 h; the expression of cyclooxygenase 2 protein was measured.

In the experiment for the effect of prostaglandin E_2 on endothelin ET_B receptor expression in macrophages, cells were stimulated by prostaglandin E_2 (10^{-8} to 10^{-5} M) alone or lipopolysaccharide plus prostaglandin E_2 for 12 h; the expression of endothelin ET_B receptor protein was measured.

2.3. Western blot

Macrophage protein (40 μg) was separated by electrophoresis on 7.5% sodium dodecyl sulfate-polyacrylamide gels and transferred to hybond polyvinylidene difluoride membranes (Amersham, Tokyo, Japan). Cyclooxygenase 2 and endothelin ET_B receptors were detected with rabbit polyclonal antiserum against cyclooxygenase 2 and human polyclonal antiserum against endothelin ET_B receptor. The protein bands were visualized by the enhanced chemiluminescence detection system (Amersham) using Kodak X-AR film. Determination of cyclooxygenase 2 and endothelin ET_B receptors was performed by densitometric analysis with a densitometer (Bio-Rad, Tokyo, Japan).

The specificity of the antibody used for immunodetection of cyclooxygenase 2 was determined in the presence of two purified forms of prostaglandin synthetase (sheep placenta) purchased from Cayman. Rabbit polyclonal antiserum against human and murine cyclooxygenase 2 (72–74 kDa) did not cross-react with murine cyclooxygenase 1 (68 kDa). The antibody for immunodetection of endothelin ET_B receptors was determined in the presence of rabbit polyclonal antiserum against human ET_B receptors (51 kDa).

2.4. Determination of intracellular cyclic AMP concentration

Macrophages were washed once with medium and were stimulated by lipopolysaccharide (10 μg/ml) or lipopolysaccharide plus prostaglandin E_2 (10^{-6} M) for 15 min, 6 or 24 h. At each incubation time, endothelin-1 at 10^{-14} M was added to the medium. After incubation with endothelin-1 for 15 min, the medium was aspirated and 600 μl of ice-cold 0.5 M trichloroacetic acid was added, then macrophages were extracted on ice for 20 min. Thereafter, they were scraped off, and the material was transferred to eppendorf tubes. The samples were then sonicated and centrifuged ($10\,000 \times g$, 5 min). The trichloroacetic acid was removed by extraction with water-saturated diethyl ether using five volumes of ether to one volume of the supernatant, and this process was repeated three times. Then, the residual ether was removed by heating the samples to 70°C for 5 min and the cyclic AMP concentration in the supernatants was measured by using an enzyme-linked immunoassay kit.

2.5. Statistical analysis

Data are expressed as means ± S.D. One-way analysis of variance was used to determine group differences. If the group values were statistically significant ($P < 0.05$), post hoc analyses were conducted using the Fisher's Protected Least-Significant Difference test.

3. Results

3.1. Promotive effects of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 expression and prostaglandin E_2 production

We previously reported the promotive effects of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 expression and prostaglandin E_2 production (Shimada et al., 1998). We confirmed these effects here for comparison with the effects of endothelin-1 in the presence of prostaglandin E_2. Endothelin-1 at 10^{-15} to 10^{-8} M promoted lipopolysaccharide-induced cyclooxygenase 2 protein expression by 35% to 54% after 12 h stimulation compared with that measured without endothelin-1 (Fig. 1), though the levels were not always dependent on the concentrations of endothelin-1. Lipopolysaccharide-induced prostaglandin E_2 production was also increased in the presence of endothelin-1 at 10^{-14} to 10^{-8} M at 12-h stimulation (Fig. 1b). This promotion appeared to decrease at concentrations of endothelin-1 higher than 10^{-12} M. As reported previously, endothelin-1 at more than 10^{-12} M induces cyclooxygenase 2 expression by itself (Shimada et al., 1998).

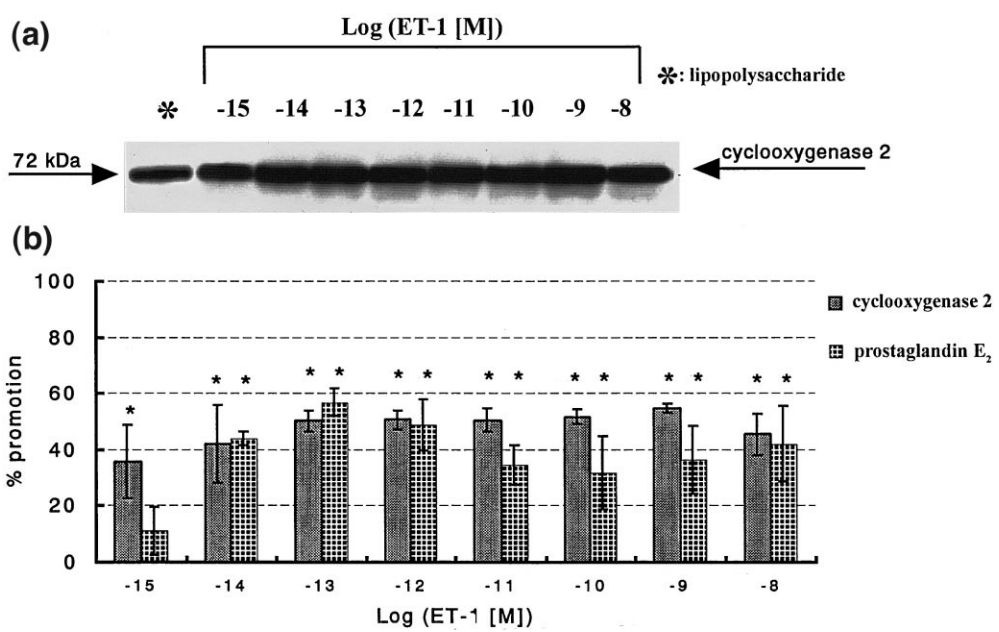

Fig. 1. The promotive effects of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 protein expression and prostaglandin E_2 production. (a) shows representative experiments of cyclooxygenase 2 protein expression after 12 h of lipopolysaccharide (10 μg/ml) stimulation with or without endothelin-1 at concentrations between 10^{-15} and 10^{-8} M. Cyclooxygenase 2 protein expression was determined by Western blot as described in Material and methods. (b) shows the promotion by endothelin-1 of cyclooxygenase 2 protein expression and prostaglandin E_2 production relative to the effect of lipopolysaccharide stimulation alone for 12 h. Cyclooxygenase 2 protein band and prostaglandin E_2 production were analyzed by densitometry and enzyme-linked immunoassay kit, respectively. Values are expressed as means ± S.D. of five independent experiments. * indicates a statistically significant difference between cells stimulated by lipopolysaccharide and lipopolysaccharide plus endothelin-1 at $P < 0.01$. Abbreviation in figure: ET for endothelin.

Therefore, when the concentrations of endothelin-1 are higher than 10^{-12} M, lipopolysaccharide-induced prostaglandin E_2 production may be enhanced by not only promotive but also additive effects of endothelin-1, and it may reach a peak level at an earlier stage and then be decreased after 12 h stimulation.

3.2. Effect of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 protein expression in the presence of prostaglandin E_2

The stimulatory effect of lipopolysaccharide alone and of lipopolysaccharide plus endothelin-1 on cyclooxygenase 2 expression was investigated. Compared to the response of prostaglandin E_2 on lipopolysaccharide-induced cyclooxygenase 2 expression, prostaglandin E_2 (10^{-8} to 10^{-5} M) inhibited cyclooxygenase 2 expression induced by lipopolysaccharide plus endothelin-1 not only concentration-dependently, but also more profoundly (Fig. 2b).

3.3. Effect of BQ788 on the opposite modulation by endothelin-1 of lipopolysaccharide-induced cyclooxygenase 2 expression

The endothelin ET_B receptor selective antagonist, BQ788 at 10^{-7} M, had no effects on lipopolysaccharide-induced cyclooxygenase 2 expression (Fig. 3, compared column A and B). The promotive effect of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 expression was inhibited by BQ788 at 10^{-7} M (Fig. 3, compared column A, C, and D), which was consistent with our previous data (Shimada et al., 1999). In addition, BQ788 at 10^{-7} M inhibited the suppression by endothelin-1 at 10^{-14} M of lipopolysaccharide-induced cyclooxygenase 2 expression in the presence of prostaglandin E_2 at 10^{-6} M (Fig. 3, compared column C, E, and F). BQ788 could not inhibit fully this opposite modulation by endothelin-1. We cannot explain this because higher concentrations of BQ788 were not effective (data not shown), and thus endothelin ET_B receptors which are less sensitive to BQ788 may also be involved in this mechanism.

3.4. Effects of prostaglandin E_2 on the endothelin ET_B receptor protein expression in macrophages

Western blot analysis using anti-human endothelin ET_B receptor antibody showed broad bands of 51 kDa molecular weight which was consistent with previous reports (Hiraki et al., 1997; Shimada et al., 1999). The amount of this protein was minimal and did not change during culture without any stimulation. Lipopolysaccharide at 10 μg/ml up-regulated endothelin ET_B receptor protein expression

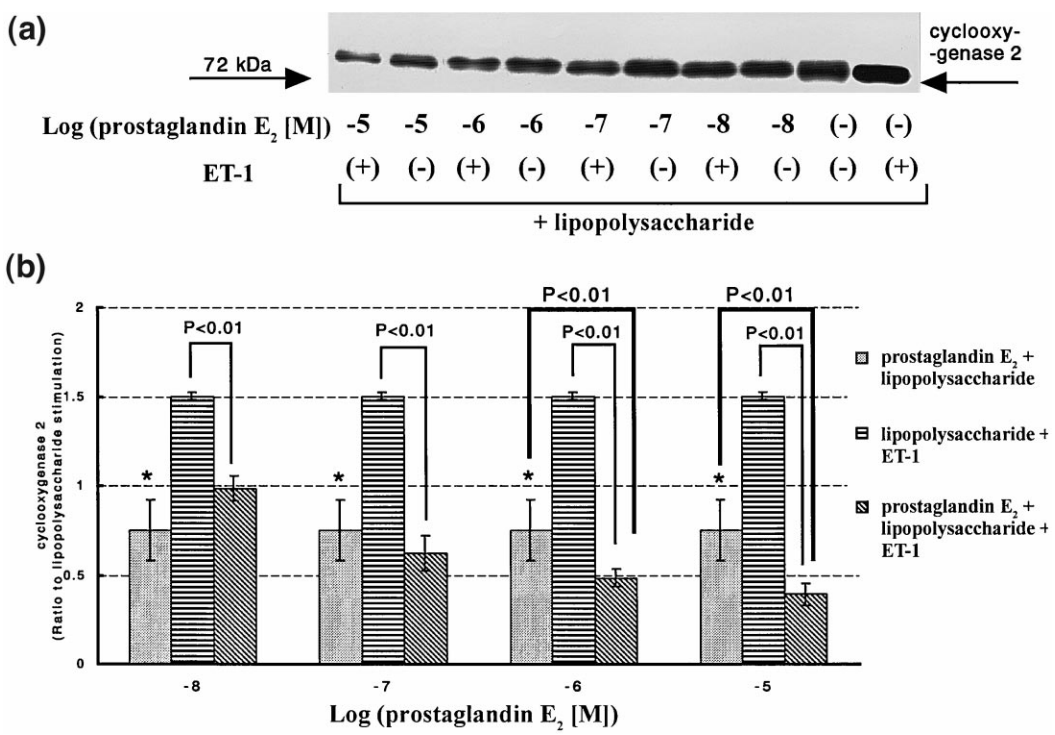

Fig. 2. Effects of endothelin-1 on lipopolysaccharide-induced cyclooxygenase 2 protein expression in macrophages in association with prostaglandin E_2. (a) shows representative experiments of cyclooxygenase 2 protein expression which was determined by Western blot as described in Material and methods. Peritoneal macrophages were prepared and treated with lipopolysaccharide (10 μg/ml) alone, lipopolysaccharide plus endothelin-1 (10^{-14} M) or lipopolysaccharide plus endothelin-1 plus prostaglandin E_2 (10^{-8} to 10^{-5} M) for 12 h, then processed for Western blotting. (b) shows the densitometric analysis of the cyclooxygenase 2 protein band which is expressed as a ratio to the value after lipopolysaccharide stimulation alone for 12 h. Values are expressed as means ± S.D. of five independent experiments. * indicates a statistically significant difference between control and lipopolysaccharide-stimulated cells at $P < 0.05$. Abbreviation in figure: ET for endothelin.

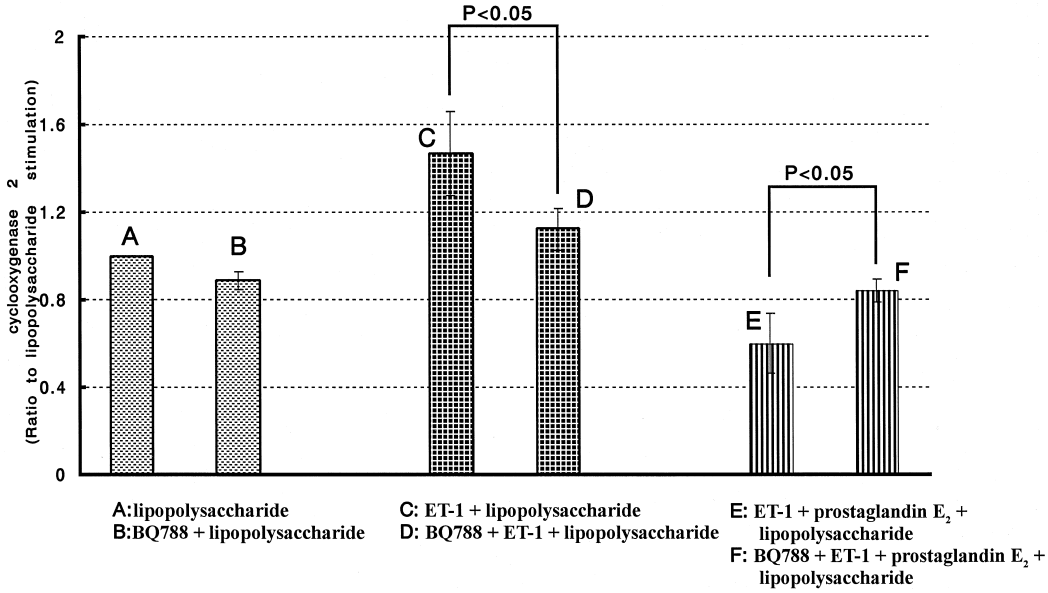

Fig. 3. The effect of BQ788 (10^{-7} M) on the modulation by endothelin-1 of lipopolysaccharide-induced cyclooxygenase 2 protein expression. Peritoneal macrophages were pretreated with the endothelin ET_B receptor selective antagonist, BQ788 at 10^{-7} M, for 30 min and stimulated by lipopolysaccharide (10 μg/ml): column B, lipopolysaccharide plus endothelin-1 (10^{-14} M): column D, or lipopolysaccharide plus endothelin-1 plus prostaglandin E_2 (10^{-6} M): column F for 12 h, or stimulated by these agents for 12 h without pretreatment with BQ788 (column A, C, and E). These cells were processed for Western blotting as described in Material and methods. The densitometric results of cyclooxygenase 2 protein band are expressed as ratios to the values of lipopolysaccharide stimulation for 12 h. Values are expressed as means ± S.D. of five independent experiments. Abbreviation in figure: ET for endothelin.

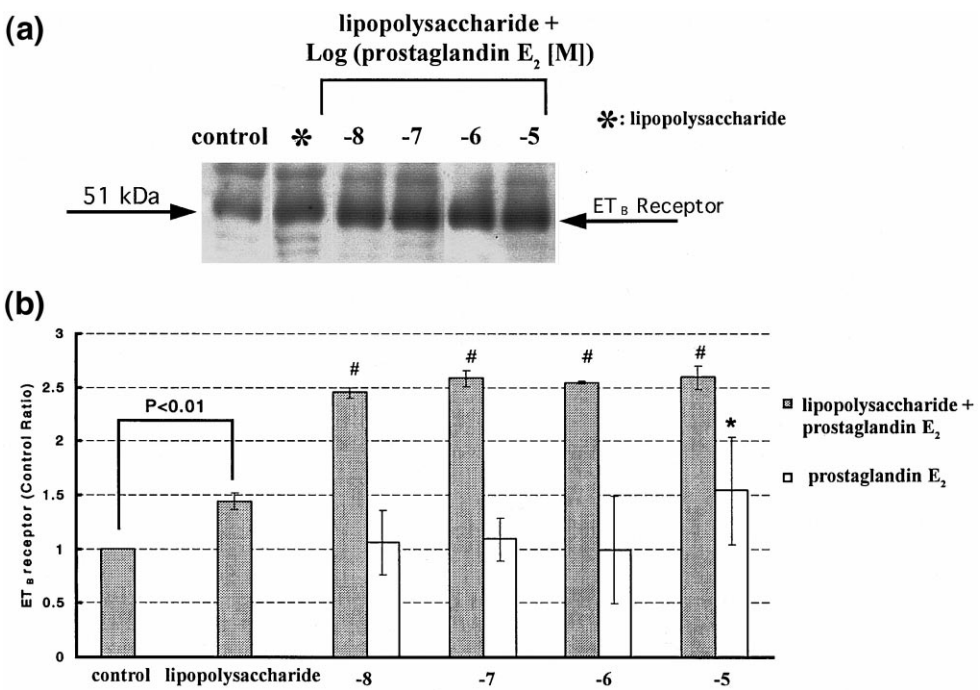

Fig. 4. Effects of prostaglandin E_2 on the endothelin ET_B receptor protein expression in macrophages. (a) shows representative experiments of endothelin ET_B receptor protein band as determined by Western blot as described in Material and methods. Peritoneal macrophages were prepared and stimulated by lipopolysaccharide (10 μg/ml), lipopolysaccharide plus prostaglandin E_2 (10^{-8} to 10^{-5} M) or prostaglandin E_2 alone for 12 h. Then the cells were processed for Western blotting. (b) shows the densitometric results of endothelin ET_B receptor band in macrophages stimulated by lipopolysaccharide plus prostaglandin E_2 (10^{-8} to 10^{-5} M) [the closed column] or prostaglandin E_2 alone [the open column] for 12 h, expressed as ratios to the values without any stimulation (control). Values are expressed as means ± S.D. of five independent experiments. * indicates a statistically significant difference between control and cells stimulated by prostaglandin E_2 at $P < 0.05$. # indicates a statistically significant difference between cells stimulated by lipopolysaccharide and lipopolysaccharide plus prostaglandin E_2 at $P < 0.01$. Abbreviation in figure: ET for endothelin; ET_B for endothelin ET_B.

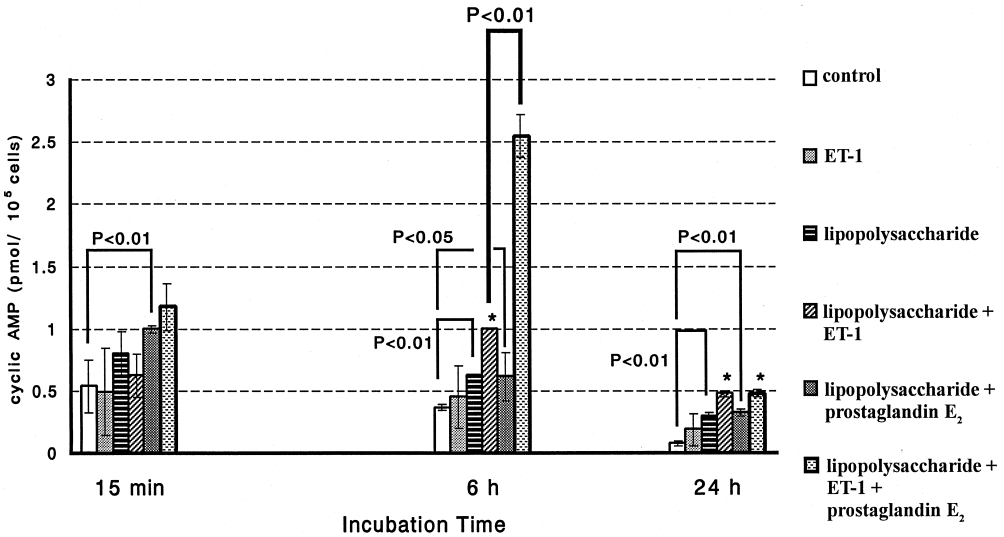

Fig. 5. Changes in cyclic AMP levels elicited by endothelin-1 in macrophages stimulated by lipopolysaccharide or lipopolysaccharide plus prostaglandin E_2. Peritoneal macrophages were seeded in 12-well plates at a density of 1.0×10^5 cells/well and stimulated by lipopolysaccharide (10 μg/ml) or lipopolysaccharide plus prostaglandin E_2 (10^{-6} M) for 15 min, 6 and 24 h. At each incubation time, endothelin-1 (10^{-14} M) was added to the medium and incubated for 15 min, and then the cells were processed for the measurement of intracellular cyclic AMP as described in Material and methods. Values are expressed as the means ± S.D. of four independent experiments. * indicates a statistically significant difference in the cyclic AMP value between cells with treatment and no-treatment of endothelin-1 for 15 min at $P < 0.01$. Abbreviation in figure: ET for endothelin; ET_B for endohelin ET_B.

1.5-fold after 12 h stimulation compared with that measured without any stimulation (control) (Fig. 4). In addition, prostaglandin E_2 at 10^{-8} to 10^{-5} M increased lipopolysaccharide-induced endothelin ET_B receptor protein expression about 1.6-fold that was independent of its concentration. Prostaglandin E_2 alone had no significant effects on the regulation of endothelin ET_B receptors except at its highest concentration, 10^{-5} M (Fig. 4b, open column).

3.5. Effects of endothelin-1 on the changes of intracellular cyclic AMP in macrophages

At first, in order to estimate the effects of endothelin-1 on intracellular cyclic AMP in macrophages, the progressive changes in cyclic AMP levels during a 24-h stimulation with lipopolysaccharide (10 μg/ml) or lipopolysaccharide plus prostaglandin E_2 (10^{-6} M) were determined. Intracellular cyclic AMP was significantly elevated in macrophages treated with lipopolysaccharide plus prostaglandin E_2 for 15 min, 6 or 24 h, or treated with lipopolysaccharide for 24 h, but no changes were observed in other cells. Treatment with endothelin-1 at 10^{-14} M (15 min) had no effects on intracellular cyclic AMP in macrophages without any stimulation. However, it significantly increased the cyclic AMP concentration in macrophages stimulated by lipopolysaccharide or lipopolysaccharide plus prostaglandin E_2 (10^{-6} M) for 6 and 24 h (Fig. 5). Particularly, this elevation was marked in macrophages stimulated by lipopolysaccharide plus prostaglandin E_2 for 6 h, which was statistically different from that in cells stimulated by lipopolysaccharide alone.

4. Discussion

A lot of reports have demonstrated the role of endothelin-1 on cyclooxygenase 2 induction and the biosynthesis of prostanoids in human hepatic stellate cells (Mallat et al., 1996), mesangial cells (Coroneos et al., 1997), and osteoblastic cells (Leis et al., 1998). Recently we have reported that high concentrations of endothelin-1 (more than 10^{-12} M) stimulate cyclooxygenase 2 expression and prostaglandin E_2 production through mainly endothelin ET_B receptors in macrophages. We also reported that lower concentrations of endothelin-1 (10^{-14} to 10^{-12} M) promoted lipopolysaccharide-induced cyclooxygenase 2 expression and prostaglandin E_2 production through endothelin ET_B receptors in macrophages, although it had no effects on the cells by itself. Compared to the response to prostaglandin E_2 (12 h incubation) on lipopolysaccharide-induced cyclooxygenase 2 expression, prostaglandin E_2 (10^{-8} to 10^{-5} M) inhibited cyclooxygenase 2 expression induced by prostaglandin E_2 has been recently demonstrated (Pang and Hoult, 1997). This was confirmed in the present study. Prostaglandin E_2 at higher concentrations than 10^{-8} M suppressed lipopolysaccharide-induced cyclooxygenase 2 protein expression. In addition, we found that prostaglandin E_2 at 10^{-8} to 10^{-5} M suppressed the promotive increase by endothelin-1 (10^{-14} M) of lipopolysaccharide-induced cyclooxygenase 2 expression in a concentration-dependent manner. It is of interest that, at high concentrations of prostaglandin E_2 (10^{-6} to 10^{-5} M), this suppression was marked in the presence of endothelin-1 (10^{-14} M) than in the absence of it. These results and previous reports suggest that endothelin-1 exerts an oppo-

site modulation of lipopolysaccharide-induced cyclooxygenase 2 expression in macrophages depending on the level of extracellular prostaglandin E_2.

This opposite modulation by endothelin-1 was mediated through endothelin ET_B receptors which were up-regulated by lipopolysaccharide or lipopolysaccharide plus prostaglandin E_2. However, in the present study, the endothelin ET_B receptor selective antagonist, BQ788, could not fully inhibit both suppressive and promotive effects. Because higher concentrations of BQ788 (more than 10^{-7} M) were not that effective (data not shown), up-regulated endothelin ET_B receptors may consist of BQ788 insensitive receptors as well as sensitive ones. In addition, although endothelin ET_B receptors were also up-regulated even at low concentrations of prostaglandin E_2 (10^{-8} to 10^{-7} M), the promotion by endothelin-1 of prostaglandin E_2-induced action was not observed. This result means that there are mechanisms other than endothelin ET_B receptor up-regulation and its related process in this modulation by endothelin-1. However, we cannot explain other mechanisms, and further research should be carried out.

There are a lot of investigations about the signal cascade following the stimulation of endothelin receptors (Karne et al., 1993; Sokolovsky, 1995) including cyclic AMP and its dependent pathway in various tissues and cells (Rubanyi and Polokoff, 1994; Chu et al., 1996; El-Mowafy and Abou-Mohamed, 1996; Mallat et al., 1996; Rebsamen et al., 1997). We studied here the changes in intracellular cyclic AMP concentrations elicited by endothelin-1 in macrophages. Endohelin-1 at 10^{-14} M markedly elevated intracellular cyclic AMP in macrophages stimulated by lipopolysaccharide and lipopolysaccharide plus prostaglandin E_2 for 6 h, especially in the latter case. This stimulation time corresponded to the time of initiation of lipopolysaccharide-induced endothelin ET_B receptor up-regulation in macrophages (Shimada et al., 1999). In addition, prostaglandin E_2 alone elevated intracellular cyclic AMP in macrophages (1.2 ± 0.2 pmol/10^5 cells) after a 15-min incubation, but it had no effect after 6 or 24 h incubation (data not shown). Therefore it is considered that endothelin-1 increased intracellular cyclic AMP in macrophages through endothelin ET_B receptors which were up-regulated by lipopolysaccharide or lipopolysaccharide plus prostaglandin E_2.

Moreover, the opposite modulation by endothelin-1 of lipopolysaccharide-induced cyclooxygenase 2 expression may depend on the levels of endothelin ET_B receptor expression and intracellular cyclic AMP elevation. Pang and Hoult (1996, 1997) showed that cyclic AMP elevating agents such as prostaglandin E_2 repress lipopolysaccharide-induced cyclooxygenase 2 expression and its activity. In contrast, Inoue et al. (1995) showed that cyclic AMP and its response element are key factors for the expression of human and mouse cyclooxygenase 2 gene. Thus, the interactions of cyclic AMP and cyclooxygenase 2 activation in lipopolysaccharide-stimulated macrophages have been confused. This problem is complex, as evidenced by the present results. In the presence of prostaglandin E_2 at high concentrations, the endothelin ET_B receptor up-regulation and cyclic AMP elevation induced by endothelin-1 of lipopolysaccharide-stimulated macrophages were marked, when endothelin-1 suppressed lipopolysaccharide-induced cyclooxygenase 2 expression. In contrast, lipopolysaccharide stimulation alone produced a mild elevation of endothelin ET_B receptors and endothelin-1-induced cyclic AMP, when endothelin-1 promoted lipopolysaccharide-induced cyclooxygenase 2 expression. We could not determine how the cyclic AMP elevation following endothelin-1 stimulation affects the regulation of cyclooxygenase 2 expression, the details of which should be further studied.

In summary, it is concluded that endothelin-1 has a promotive or suppressive effect on lipopolysaccharide-induced cyclooxygenase 2 protein expression through endothelin ET_B receptors according to the levels of extracellular prostaglandin E_2. This evidence suggests that endothelin-1 is an important agent in lipopolysaccharide-induced inflammatory responses in macrophages.

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